

Sexual Dimorphism of Basitarsi in Pest Species of *Diabrotica* and *Cerotoma* (Coleoptera: Chrysomelidae)

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ABSTRACT Sexual dimorphism in basitarsal pad morphology is described for prothoracic and mesothoracic legs of *Diabrotica virgifera virgifera* LeConte, *Diabrotica barberi* Smith & Lawrence, and *Diabrotica undecimpunctata howardi* Barber (Coleoptera: Chrysomelidae) and for prothoracic legs of *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae). On the indicated legs of these diabroticite beetles, the typical structure of the hairy tarsal pads of chrysomelid beetles is replaced proximally on tarsomere I of males by a hairless planar ovoid patch that may facilitate maintenance of the mating posture of males upon the elytra of females. This basitarsal patch proved as reliable as the standard supra-anal plate character for establishing sex of these important crop pests.

KEY WORDS corn rootworm beetle, bean leaf beetle, basitarsal pad, sexual dimorphism

Diabroticites are New World chrysomelid beetles (Coleoptera) that include several significant pests of North American agriculture. In the United States, the most destructive diabroticites are the western, northern, and southern corn rootworm, *Diabrotica virgifera virgifera* LeConte, *Diabrotica barberi* Smith & Lawrence, and *Diabrotica undecimpunctata howardi* Barber, respectively (Krysan 1986, Metcalf 1986). Corn rootworm damage is most likely in maize, *Zea mays* L., although *D. u. howardi* injures additional crops, including assorted cucurbits and legumes (Krysan 1986, Metcalf 1986). Another diabroticite pest, the bean leaf beetle, *Cerotoma trifurcata* (Forster), feeds on a variety of leguminous host plants (Kogan et al. 1980).

The economic importance of these pests and widespread use of insecticides to control especially the corn rootworms has led to considerable study of their biology. Other factors that have sustained interest in these diabroticites include repeated emergence of corn rootworm populations able to circumvent conventional management strategies based on crop rotation and insecticides (Krysan et al. 1986, Meinke et al. 1998, Levine et al. 2002); the need for improved resistance management strategies for preservation of maize genetically modified to produce *Bacillus thuringiensis* Berliner proteins toxic to corn rootworm larvae (Bates et al. 2005); the corn rootworm areawide management program exploiting semiochemicals to enhance specificity of insecticidal baits (Chandler et al. 2000); and the increasing importance of *C. trifurcata* in the upper Midwest as a pest of soybean, *Glycine*

max (L.) Merr. (Krell et al. 2004). The introduction and spread of *D. v. virgifera* in Europe also has stimulated study overseas (Rasmann et al. 2005).

Much biological research requires that the sex of experimental insects be determined. Test design, time constraint, or poor condition of field-trapped insects often precludes sacrifice of specimens for dissection and examination of internal reproductive morphology. The most reliable external character for separating the sexes of *Diabrotica* adults is the presence in males of a supra-anal plate, an extra sclerite at the posterior abdominal tip (Smith and Allen 1931, White 1977). *C. trifurcata* sexes can be distinguished in a similar manner (Eddy and Nettles 1930), although a much darker frons in females than males provides an easier means of sex determination for this species (Horn 1893, Kogan et al. 1980). Sex of some diabroticites also can be determined from antennal morphology, although differences can be subtle (Bechyné 1956, Krysan 1986, Cabrera and Cabrera Walsh 2004). In *D. v. virgifera*, the pattern of elytral vittae varies with sex but is unreliable for establishing gender of individual specimens (Kuhar and Youngman 1995, Hesler and Hammack 1997). Sexual dimorphism in diabroticite basitarsal morphology also occurs (Bechyné 1956, Cabrera and Cabrera Walsh 2004), but it is little known outside the taxonomic literature. Here, we highlight the presence in males of ventral planar basitarsal patches that are likely adhesive in function and demonstrate their reliability for determining the sex of *D. v. virgifera*, *D. barberi*, *D. u. howardi*, and *T. trifurcata* adults.

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Materials and Methods

Diabrotica v. virgifera and *D. barberi* adults were collected in maize in 2004, except that specimens for photography were taken from the rearing facility at

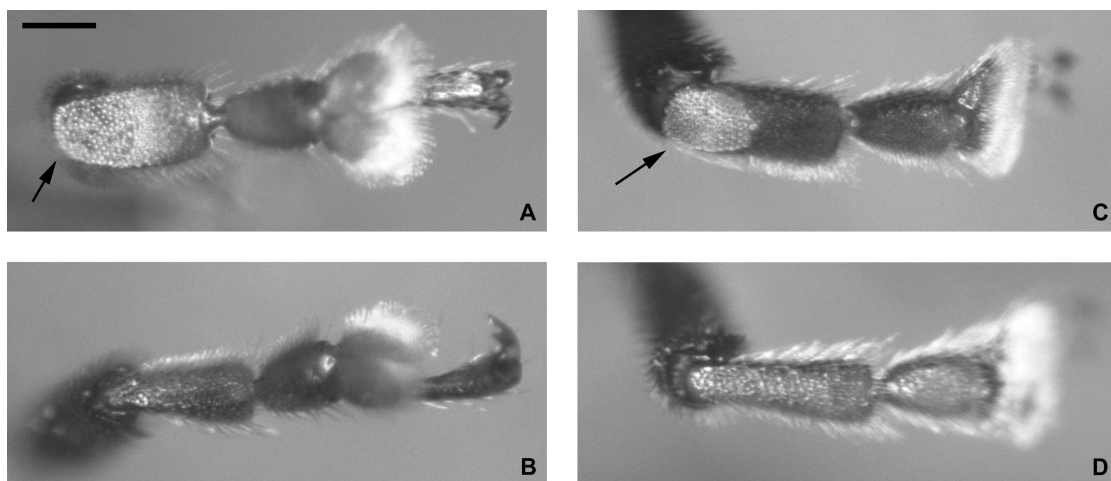


Fig. 1. Stereomicrographs of *D. v. virgifera* tarsi in ventral view, with arrows indicating planar patch on tarsomere 1 of males. Prothoracic tarsus of male (A) and female (B). Mesothoracic tarsus of male (C) and female (D). Scale bar (A) = 200 μ m and applies to all Fig. 1 photographs.

the Brookings laboratory, where they were colonized on maize (Jackson 1986, and references therein). Adults of *D. u. howardi* were gathered in cucurbits in 2003–2004. *C. trifurcata* was obtained in soybean in 2001–2004. All field collections occurred in Brookings County, SD.

Sex of 100 adult specimens per species was determined using both the structure of basitarsi on legs separated from the thorax and from abdominal morphology by using the presence or absence of the supranal plate (Smith and Allen 1931, White 1977). Isolated legs and legless specimens were coded so that sex determinations by using each criterion could be done independently. One worker coded the specimens, removed their legs under magnification too low for observation of relevant basitarsal structure and deter-

mined sex of the legless specimens by using the supranal plate method. Another worker determined sex based on basitarsal structure of the isolated coded legs.

Photography was done on a Zeiss MC 80 camera mounted on a Zeiss Stemi SV 6 stereomicroscope. The latter was used for all morphological observations.

Results

The sexually dimorphic tarsal character of primary interest is a hairless planar ovoid patch located proximally on the ventral surface of the basitarsus (first tarsomere) of males but not females. This specialization occurs on prothoracic and mesothoracic legs of the *Diabrotica* species examined, but only prothoracic legs of *C. trifurcata*. Even in the test *Diabrotica*, the

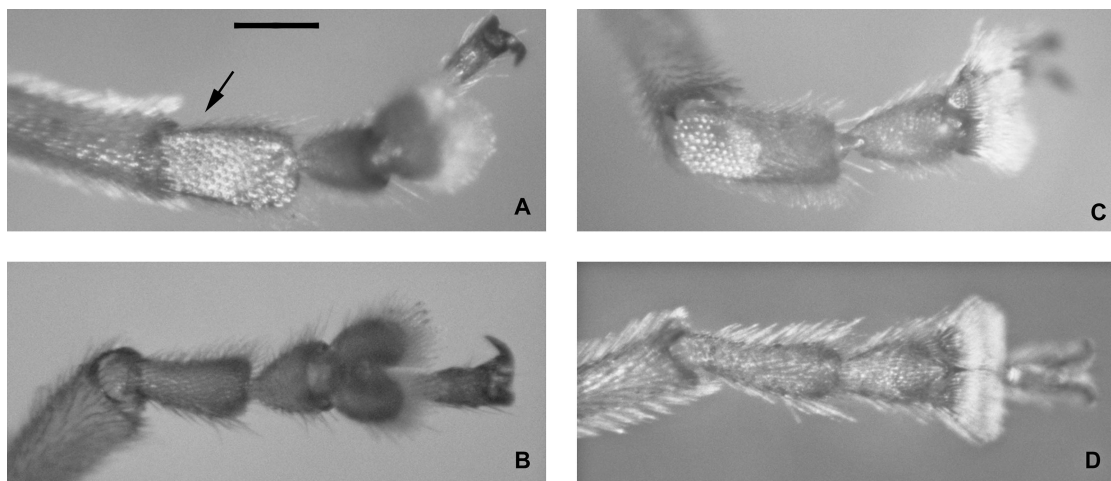


Fig. 2. Stereomicrographs of *D. barberi* tarsi in ventral view, with arrows indicating planar patch on tarsomere 1 of males. Prothoracic tarsus of a male (A) and female (B). Mesothoracic tarsus of a male (C) and female (D). Scale bar (A) = 200 μ m and applies to all Fig. 2 photographs.

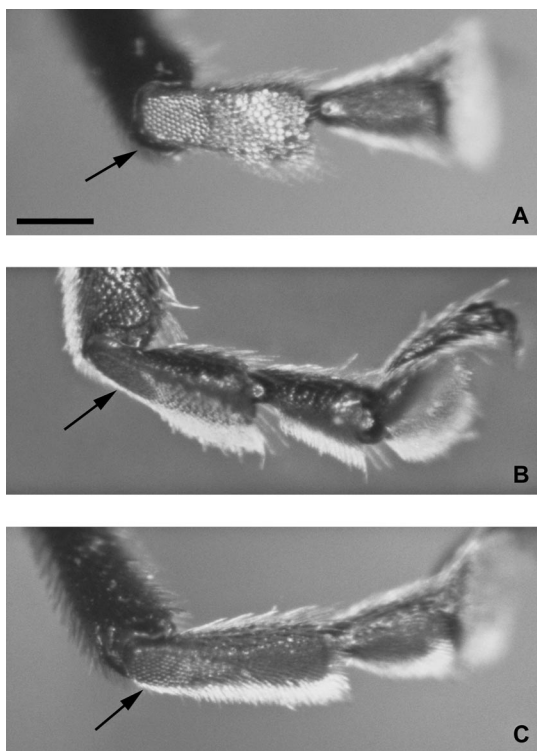


Fig. 3. Stereomicrographs of tarsi of *D. undecimpunctata howardi* males, with arrows indicating planar patch on tarsomere 1. Prothoracic tarsus in ventral (A) and side (B) views. Mesothoracic tarsus in ventral (C) view. Scale bar (A) = 200 μ m and applies to all Fig. 3 photographs.

patches are somewhat larger on the prothoracic than the mesothoracic legs.

These patches cover at least half of the ventral surface of the prothoracic basitarsi in adult males of both *D. v. virgifera* and *D. barberi* (Figs. 1A and 2A, respectively). Each prothoracic patch abuts a field of stocky discoid setae that are situated more distally on the tarsomere and illustrated more clearly for *D. barberi* (Fig. 2A) than for *D. v. virgifera* (Fig. 1A). Female beetles of both species lack analogous prothoracic patches and the discoid setae of the male (Figs. 1B and 2B for *D. v. virgifera* and *D. barberi*, respectively). Note also the stockiness of the prothoracic basitarsus of the male, especially the proximal region, compared with the same area of the female in both *D. v. virgifera* and *D. barberi* (Fig. 1A versus B and 2A versus B, respectively). On the mesothoracic legs of *D. v. virgifera* and *D. barberi* males, the tarsal patches are nearly half as long as the first tarsomere (Figs. 1C and 2C, respectively), which seems to lack the extensive associated field of discoid setae found more distally on the prothoracic basitarsi of these species. Again, analogous mesothoracic tarsal patches are absent in the female (Figs. 1D and 2D for *D. v. virgifera* and *D. barberi*, respectively).

The basitarsal patches in *D. u. howardi* males are relatively small compared with those of *D. v. virgifera*

and *D. barberi*. Those on the prothoracic basitarsi cover about one-third of the length of the ventral surface of the tarsomere; however, an adjacent field of stocky discoid setae extending distally over the rest of the ventral surface of the tarsomere is particularly obvious in *D. u. howardi* (Fig. 3A). Note the hairy appearance of the distal ventral portion of the prothoracic basitarsus when viewed from the side, in contrast with the proximal region occupied by the planar patch (Fig. 3B). The patches are reduced even further on the mesothoracic basitarsus of *D. u. howardi* males, where they span $\approx 25\%$ of the length of its ventral surface (Fig. 3C). A small triangle apparently containing discoid setae abuts the distal end of the mesothoracic basitarsal patch (Fig. 3C). The basitarsus of the prothoracic and mesothoracic legs of females lacks the patch present in males (females not shown).

Analogous planar patches on the prothoracic basitarsus of *C. trifurcata* males cover about two-thirds of its ventral surface (Fig. 4A). Females lack similar patches (Fig. 4B). The discoid setae prominent in *D. u. howardi* males are not obvious on the prothoracic basitarsus in either sex of *C. trifurcata*.

The planar basitarsal patches of all of the species examined here seem sculptured at regular intervals. This sculpturing is evident, for example, on the prothoracic patch of *D. barberi* (Fig. 2A) and *C. trifurcata* (Fig. 4A) and on a higher magnification view of the mesothoracic patch of *D. v. virgifera* (Fig. 5), where the distance between pattern repeats is $\approx 20 \mu$ m.

Identical determinations of sex were obtained in all four test species by using the basitarsal patch method and the standard method based on the supra-anal plate.

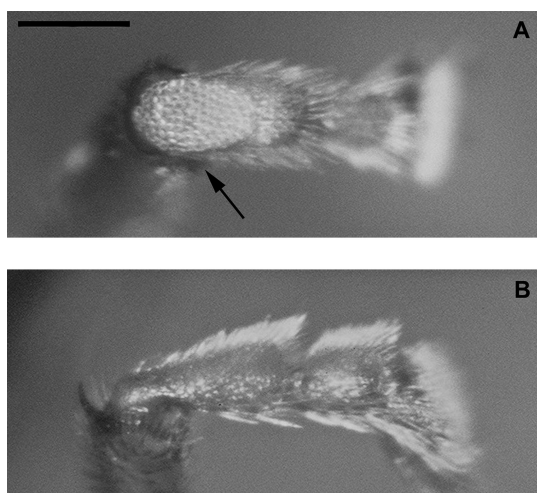


Fig. 4. Stereomicrographs of *C. trifurcata* tarsi, with arrow indicating planar patch on prothoracic tarsomere 1 of a male. Prothoracic tarsus of male (A) in ventral view and female (B) in ventrolateral view. Scale bar (A) = 200 μ m and applies to both Fig. 4 photographs.

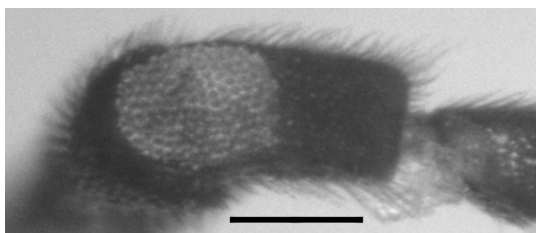


Fig. 5. Stereomicrograph of mesothoracic tarsomere 1 of *D. virgifera virgifera* highlighting the sculptured appearance of the ventral planar patches of diabroticite males. Scale bar = 200 μ m.

Discussion

The tarsi of Chrysomelidae typically bear five tarsomeres, although the fourth tarsomere is reduced and hidden in the bilobed third tarsomere characteristic of the family (Arnett 1963). Also typical of the family are large ventral tarsal pads bearing adhesive hairs that likely aid attachment and locomotion on aerial portions of host plants (Stork 1983). Tarsal specialization for sexual function is widespread in chrysomelids, often with prothoracic and sometimes mesothoracic modification occurring in the male (Crowson 1981). The first prothoracic tarsomere of flea beetles, for example, is slightly wider in males than females (Konstantinov 2002) and is dilated in males of a few *Diabrotica* species (Horn 1893). Although Horn (1893) listed *D. virgifera* among the species without dilated tarsi, we noted thickened male basitarsi on prothoracic legs of *D. virgifera*, *D. barberi*, and *C. trifurcata* and on mesothoracic legs of the two *Diabrotica* species. Along with stockier tarsomeres, the ventral hairy tarsal pads typically present in both chrysomelid sexes may often, in the male, incorporate discoid setae, which are stocky adhesive hairs specialized for grasping the female during copulation (Stork 1983, and references therein). The discoid setae evident here in *Diabrotica* males, and especially prominent on the prothoracic legs of the *D. u. howardi*, likely function similarly.

As for structures resembling the hairless basitarsal patches of our test diabroticites, Blake (1958) noted thickened or swollen prothoracic basitarsi with "an undersurface having a flat tympanum-like appearance" in males of two other genera of Galerucini: *Leptonesiotes* and *Phyllecthrus*. Cabrera and Cabrera Walsh (2004) described a new diabroticite genus, *Platybrotica*, with a ventral adhesive patch on tarsomere one of the prothoracic and mesothoracic legs of males. Morphology of these patches was not described, but their placement matches that of the sexually dimorphic basitarsal patches reported here for the three *Diabrotica* species. Bechyné (1956) described similarly situated flat, hairless patches in *Diabrotica fucata* (F.) males and deemed them characteristics of the genus. He likened their appearance to the multifaceted surface of compound eyes, but he considered their function unknown.

The observed sexual dimorphism in diabroticite basitarsal patches is likely related to the propensity of diabroticite males to retain tarsal contact with the elytra of females during copulation (Lew and Ball 1979, Medvedev and Pavlov 1988, Tallamy et al. 2003). We observed that *D. v. virgifera* and *D. barberi* males press their prothoracic and mesothoracic basitarsi against the elytra of their sexual partners, and, because footing was often maintained without additional contact of these legs with the elytra of females despite their vigorous side-to-side rocking motions, the planar patches are likely adhesive structures that help to maintain the mating posture of males (unpublished observations). Digital video analyses of *Diabrotica* mating behaviors underway (B.W.F.) will help test this hypothesis.

Regardless of function, the basitarsal patches of males proved to be a reliable external trait for separating the sexes of *D. v. virgifera*, *D. barberi*, *D. u. howardi*, and *C. trifurcata*. We found this trait a useful character for differentiating sex of *Diabrotica* beetles caught on traps covered by adhesive (Hammack and Petroski 2004), which sometimes obscures the terminal abdominal area and renders observation of the supra-anal plate of males difficult.

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